BIOCHIMICA

BIOPHYSICA ACTA

STUDIES ON ACID HYDROLASES
I. A PROCEDURE FOR THE PREPARATION OF ACID DEOXYRIBONUCLEASE AND OTHER ACID HYDROLASES

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SUMMARY

A procedure for the preparation of spleen acid deoxyribonuclease (deoxyribonucleate 3'-oligonucleonucleotidohydrolase, EC 3.1.4.6; deoxyribonuclease II) is described. The procedure can be used, with only slight modifications, to prepare other acid hydrolases from spleen, as well as from other tissues and organs.

Two enzyme components, called A and B, have been separated by chromatographic fractionation of acid deoxyribonuclease on CM-Sephadex. The two components have the same enzymological, macromolecular and spectroscopic properties. Preliminary evidence suggests that the component, A, is derived from the minor component, B, by a deamidation process occurring during the acidic extraction of the enzyme from the tissue.

INTRODUCTION

Spleen acid deoxyribonuclease (deoxyribonucleate 3'-oligonucleotidohydrolase, EC 3.1.4.6; deoxyribonuclease II), an enzyme recently isolated as a homogeneous protein[1,2], has been the object of extensive investigations in this laboratory over the past few years. These have shown that the enzyme is able to split simultaneously both DNA strands at the same level[3-6], and is competitively inhibited by tRNA and certain synthetic polyribonucleotides[7]. Acid deoxyribonuclease has been characterized with respect to its physical and chemical properties[8], and shown to be a dimeric protein molecule, which exhibits a cooperative type of binding toward a synthetic substrate, bis(*p*-nitrophenyl)phosphate[9].

In view of the large amounts of enzyme necessary for our current investigations, we have modified the isolation procedure of BERNARDI AND GRIFFE[2] so as to reduce the labor involved and to increase the enzyme output. The new procedure to be described in the present article can be used, with only slight modifications, to pre-

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pare other acid hydrolases; these enzymes are considered to have the same lysosomal origin as acid deoxyribonuclease[10]. In fact, the two following articles describe the preparation and the properties of two such enzymes, acid phosphomonoesterase[11] and acid ribonuclease[12]. Furthermore a method for the preparation of spleen exonuclease (spleen phosphodiesterase) is in an advanced stage of development[13]. Preliminary work indicates that the present method can be used for the purification of other acid hydrolases which are also present in the crude preparation of spleen nuclease (see RESULTS); these include two ribonucleases and one phosphomonoesterase which are different from the enzymes described in the following articles[11,12], nucleoside polyphosphatase[14], lysozyme and hyaluronidase. Furthermore, the present method has also been applied to thymus and liver with results similar to those reported here for spleen, and seems to be suitable for other tissues as well. The ultimate goal of this series of investigations is to obtain a better understanding of the biological role of acid hydrolases in the living cell, as well as to obtain a number of useful enzymes to be used as tools in the study of the structure of nucleic acids.

It may be worthwhile pointing out that acid deoxyribonuclease, acid phosphomonoesterase and acid ribonuclease are, to our knowledge, the first lysosomal enzymes which have been obtained in a homogeneous state. A concise description of the procedures for the preparation of these three enzymes has been published elsewhere[15].

EXPERIMENTAL PROCEDURE

Materials and methods

These have been already described elsewhere[2,8]. DEAE–Sephadex A-50 (coarse), CM–Sephadex C-50 (medium), and Sephadex G-25 (bead form) and G-100 were used, in addition to materials already described[2], for column chromatography; these products were purchased from Pharmacia AB (Uppsala, Sweden).

Assay of enzymatic activities. The acid deoxyribonuclease assay was carried out essentially as already described[2]. The reaction mixture (2.5 ml) contained: (a) 2.4 μmoles DNA-P*, 375 μmoles acetate buffer (pH 5.0), 25 μmoles EDTA; (b) enzyme; if necessary, this was diluted with 0.15 M acetate buffer–0.01 M EDTA (pH 5.0), containing 0.05 % cytochrome c (type III or type V; Sigma Chemical Co., St. Louis, Mo.); alternatively concentrated enzyme solutions ($A_{280m\mu} = 3$–7) were added to the DNA solution in μl amounts, using Carlsberg constriction micropipettes.

After 10 min of incubation at 37° the reaction was stopped by adding 0.5 ml of 12 % perchloric acid. The mixture was then chilled in an ice-bath for 10 min and clarified by centrifugation at 4°. The extent of DNA hydrolysis was determined by measuring the absorption of the supernatant at 260 mμ. After subtracting a suitable blank, readings were corrected for dilution with the enzyme solution and the perchloric acid.

Assays were performed using enzyme concentrations so as to obtain $A_{260m\mu}$ readings in the range 1–4. Under these conditions a linear relationship was obtained

* Calf-thymus DNA was prepared according to method B of ref. 5.

between enzyme concentration and acid-soluble oligonucleotide formation. One activity unit is defined as the amount of enzyme which catalyses the liberation of oligonucleotides having a (corrected) $A_{260m\mu}$ value equal to 1 per min, under the conditions defined above. If the volumes used in the assay are halved, the activity values were divided by 2. Since the molar absorption at 260 mμ of the oligonucleotide-phosphorus present in the final completely acid-soluble digest is 9100, one activity unit corresponds to the liberation of 0.22 μmoles of oligonucleotide-phosphorus. The specific activity was calculated, unless otherwise stated, by dividing the activity by the $A_{280m\mu}$ of the enzyme solution.

Ribonuclease and exonuclease were assayed according to BERNARDI AND BERNARDI[12,13], and acid phosphomonoesterase according to CHERSI, BERNARDI AND BERNARDI[11]. The phosphodiesterase assay was carried out using bis (*p*-nitrophenyl) phosphate as the substrate, 0.25 M succinate (pH 6.4) as the solvent, 2 M$(NH_4)OH$ (+0.1 M EDTA, if the calcium salt of the substrate was used) to stop the reaction; otherwise the assay was identical to that described for acid phosphomonoesterase[11]. Under these conditions, the assay essentially detects the nucleoside polyphosphatase activity.

RESULTS

Isolation of the enzyme

As in the previous method[2], this involves: (a) the preparation of a crude enzyme and (b) its chromatographic purification.

Preparation of crude spleen nuclease I. All operations were carried out at room temperature, except where otherwise stated. Hog spleens were trimmed, ground with an electrical 1 HP meat-grinder and homogenized with 0.05 M H_2SO_4 (1 l/kg of ground spleen; 1 ml of isooctanol was added to each batch.) The pH of the homogenate was about 4.2. 0.1 M H_2SO_4 was added dropwise to the homogenate, with mechanical stirring, until a pH of 2.5 was attained; this required about 1 l of 0.1 M H_2SO_4 per kg of spleen. Solid $(NH_4)_2SO_4$ (243 g/l; 0.4 saturation at 20°) and EDTA (2.43 g/l) were then added to the suspension. This was allowed to stand overnight at 4° and then was centrifuged for 1 h at 8000 $\times g$ at 4°. Solid $(NH_4)_2SO_4$ (285 g/l; 0.8 saturation at 20°) and EDTA (2.85 g/l) were added to the supernatant from the previous step, and the suspension was stored, generally for several days, at 4°. The suspension was then filtered through a $K_0$00 Seitz filter on a Seitz press-filter (Seitz-Asbest-Werke; Bad Kreuznach, Germany). Over 60 l of suspension may be easily filtered through the same filter, under atmospheric to +0.5 kg/cm² pressure. The filtered precipitate was stored at −15°. When enough material was available, this was thawed and dissolved in a small volume of distilled water (about 10 ml/kg of spleen). The turbid solution was dialyzed against several changes of distilled water at 4° for 48 h, clarified by centrifugation, concentrated by freeze-drying, and dialyzed against 0.05 M phosphate buffer (pH 6.8). This product will be called crude spleen nuclease II, to distinguish it from crude spleen nuclease I* of the previous method[2].

* Crude spleen nuclease I is the preparation called crude enzyme in ref. 2.

Acid deoxyribonuclease activity was determined at the different steps leading to the crude enzyme preparation, and the results are shown in Table I. Two modifications of the procedure, in which 0.1 M HCl and 0.15 M NaCl, respectively, replaced 0.05 M H_2SO_4 in the extraction mixture, were also studied and the enzymatic activities are reported in Table I.

TABLE I

PREPARATION OF SPLEEN ACID DEOXYRIBONUCLEASE

Values show total activities per kg of trimmed spleen as determined on aliquots taken from a preparation at the subsequent steps indicated in the first column. The supernatants obtained by centrifuging products 1, 2, and 3 at 8000 × *g* for 1 h, and the aqueous solution of precipitate 4, were dialyzed against 0.15 M NaCl and assayed.

Preparation step	*Extraction procedure*			*Dry weight**
	0.15 *M NaCl*	0.1 *M HCl*	0.05 *M* H_2SO_4	
1. Extraction	1270	3890	3880	4.1*
2. Acidification to pH 2.5**	3170	4540	4100	2.9*
3. 0.4 $(NH_4)_2SO_4$ saturation***	2670	2940	3080	0.7*
4. 0.8 $(NH_4)_2SO_4$ saturation	3040	3600	3570	—

* This column gives the dry weight of undialyzable material per kg of trimmed spleen, as determined on the dialyzed products mentioned above (0.05 M H_2SO_4 extraction).

** This was done with 0.2 M HCl for the extracts obtained with 0.15 M NaCl and 0.1 M HCl.

*** The low values found at this step are probably due to the presence of residual SO_4^{2-}; this is an inhibitor of acid deoxyribonuclease[2].

Crude spleen nuclease II is obtained in a yield of 0.2–0.3 g (dry weight) per kg of ground spleen. Its total deoxyribonuclease activity is 2700–3000 units/kg of ground tissue, the specific activity being about 10. Slightly higher values (3200–3300 units/kg) were obtained in the most recent preparations. The $A_{280m\mu}/A_{260m\mu}$ ratio for the crude enzyme is equal to 1.3–1.5.

Chromatographic purification. A procedure (here called Procedure C) has been developed to replace the previously described methods A and B[2] in order to purify large quantities of spleen crude nuclease II. A summary of the new procedure is given in Table II which refers to one typical large-scale preparation, as does the description of the chromatographic steps which follows (see below). The figures refer to a chromatographic purification, which, although performed on columns having the same dimensions as those of the large-scale purifications, was carried out on a crude spleen nuclease II preparation obtained from 13 kg of trimmed spleen (preparation HS 9). This choice was determined by three considerations. The first was that a large number of enzymatic activities was determined during the purification of preparation HS 9. The second was that the scale of this preparation may be more readily accessible to other investigators than that of the larger preparations. The third was that preparation HS 9 was particularly rich in the A component and the CM-Sephadex chromatography therefore showed an evident first peak of deoxyribonuclease activity (*vide infra*).

TABLE II

CHROMATOGRAPHIC PURIFICATION OF SPLEEN ACID DEOXYRIBONUCLEASE (PROCEDURE C)

The reported data refer to preparation HS 11. All values quoted refer to the fractions which were processed further or to the final product; the sides of the activity peaks were processed separately. Preparation HS 11 was obtained from 60 kg of spleen.

Fraction	*Weight (g)*	*Volume (ml)*	*Total units*	*Total $A_{280m\mu}$*	*Specific activity*	
					$A_{280m\mu}$	*weight**
Crude spleen nuclease II	10	400	160 000	125 000	12.8	16
I. DEAE–Sephadex	0.935	725	105 000	1625	64.5	112
II. Hydroxyapatite	0.193	425	61 500	225	273	318
III. CM–Sephadex A**	0.0185	20	7850	22.4	350	425
CM–Sephadex B**	0.073	30	31 000	88.5	350	425

* Specific activity data given in this column were obtained by dividing the activity (total units) by the dry weights (in mg) of the enzyme preparations.

** Values reported refer to fractions A and B, respectively (see text).

Step I. DEAE–Sephadex A-50 (*Fig.* 1). 10 to 20 g of crude spleen nuclease II in 300–500 ml of 0.05 M phosphate buffer (pH 6.8) was loaded on a 8 cm × 80 cm column equilibrated with the same buffer. Under these conditions several enzymatic activities were not retained: ribonuclease (Fig. 1, ref. 12), phosphodiesterase (Fig. 1, ref. 11), acid phosphomonoesterase (Fig. 1, ref. 11), cytochrome *c*, and acid deoxyribonuclease (Fig. 1). The column was then washed with 0.5 M phosphate buffer (pH 6.8). A second large fraction was eluted which contains acid ribonuclease and acid phosphomonoesterase activities. These activities differ from those contained in the first peak[11,12].

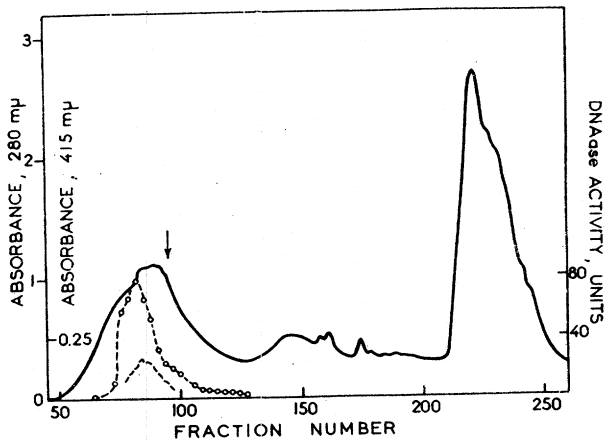

Fig. 1. Chromatography of crude spleen nuclease II on DEAE–Sephadex A-50 (Procedure C, Step I). 330 ml of preparation HS 9 ($A_{280m\mu}$ = 10.3; $A_{260m\mu}$ = 6.9) were loaded on a 8 cm × 80 cm column of DEAE–Sephadex A-50 equilibrated with 0.05 M phosphate buffer (pH 6.8). This buffer was also used to elute the first protein peak. 0.5 M phosphate buffer (pH 6.8) was loaded at the fraction indicated by the arrow. 24-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand scale). The broken line indicates the absorption at 415 mμ of cytochrome *c* (left-hand inner scale). Fractions 50–65 were processed further. Acid and basic ribonuclease, acid phosphomonoesterase and phosphodiesterase were also assayed; the results are shown elsewhere[12,11].

Step II. Hydroxyapatite (*Fig. 2*). The deoxyribonuclease-rich fractions from Step I were loaded on a 2 cm × 40 cm column equilibrated with 0.05 M phosphate buffer (pH 6.8). A molarity gradient (0.05 to 0.5) of this buffer eluted three main protein peaks. The first peak contained nucleoside polyphosphatase (Fig. 2; ref. 11) and the first ribonuclease fraction eluted from DEAE–Sephadex (Fig. 2); the second peak contained acid phosphomonoesterase (Fig. 2, ref. 11), cytochrome *c*, and the second ribonuclease fraction eluted from DEAE–Sephadex (Fig. 2); the third peak contained acid deoxyribonuclease (Fig. 2).

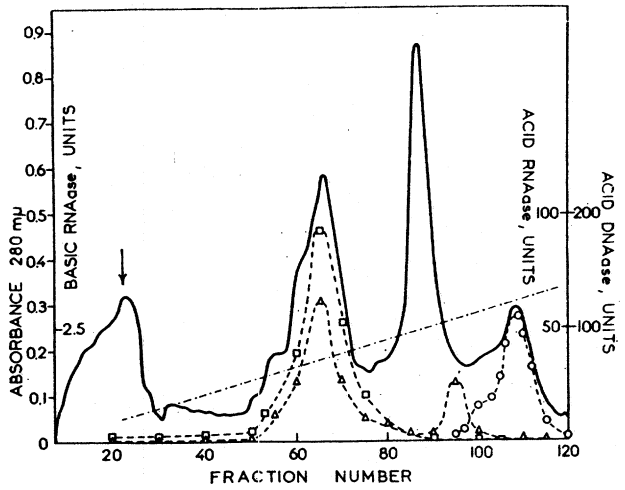

Fig. 2. Chromatography of fractions 50–65 from Step I on hydroxyapatite (Procedure C; Step II) 370 ml ($A_{280m\mu}$ = 1.48) were loaded on a 2 cm × 40 cm column of hydroxyapatite equilibrated with 0.05 M phosphate buffer (pH 6.8). A molarity gradient (0.05 to 0.5 M) was started at the fraction indicated by the arrow; at fraction 120 the molarity of the effluent was 0.35. 24-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand scale). Cytochrome *c* was eluted as a sharp peak centered on fraction 86 ($A_{415m\mu}$ = 0.82; not shown in the figure). Acid ribonuclease (squares; right-hand inner scale) and basic ribonuclease (triangles; left-hand inner scale) are also shown. Fractions 100–115 were concentrated by freeze-drying to about 70 ml, filtered through a Sephadex G-25 column equilibrated with 0.075 M phosphate buffer (pH 6.8) and processed further.

Step III. CM–Sephadex C-50 (*Fig. 3*). The deoxyribonuclease-rich fractions from the previous step were dialyzed against 0.075 M phosphate buffer (pH 6.8) and loaded on a 2 cm × 100 cm column equilibrated with 0.05 M phosphate buffer (pH 6.8). Elution was then carried out with a molarity gradient (0.1 to 0.4 M) of phosphate buffer (pH 6.8). Acid deoxyribonuclease activity was eluted at a fairly constant specific activity in two peaks, at about 0.18 M and 0.22 M phosphate, respectively. The first, minor, component, which in most cases appeared as a shoulder of the main peak, will be called deoxyribonuclease A; the second, major, component will be called deoxyribonuclease B.

The central parts of the two peaks were re-run separately on 1 cm × 100 cm CM–Sephadex columns, after adjusting the phosphate molarity in the solvent to

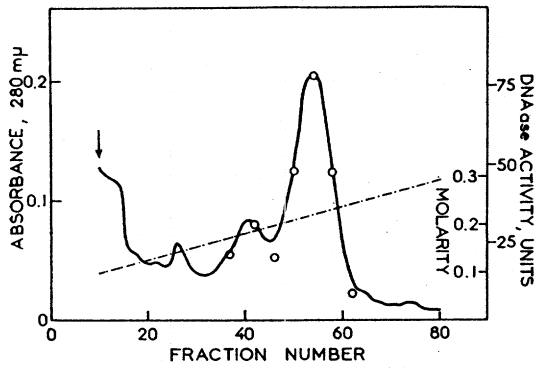

Fig. 3. Chromatography of fractions 100–115 from Step II on CM–Sephadex C-50 (Procedure C; Step III). 115 ml ($A_{280m\mu}$ = 0.490) were loaded on a column equilibrated with 0.05 M phosphate buffer (pH 6.8). A molarity gradient (0.1 to 0.4 M) of phosphate buffer (pH 6.8) was started at the fraction indicated with an arrow (right-hand inner scale). 11-ml fractions were collected. The continuous line indicates the absorption at 280 mμ. Circles indicate the acid deoxyribonuclease activity (right-hand outer scale). Fractions 37–48 and 49–61 were processed further.

0.075 by dialysis or Sephadex G-25 gel filtration. Upon rechromatography, each fraction was eluted as a single peak (Fig. 4) at the same phosphate molarities as in the first CM-Sephadex chromatography, with a constant specific activity of about 350.

The central parts of the rechromatographed peaks were loaded on Sephadex G-25, equilibrated with 0.001 M acetate buffer (pH 5.0). The enzyme fractions were then concentrated by freeze-drying to solutions having $A_{280m\mu}$ values $\geqq$ 3.0, frozen, and stored at −60°. These fractions were referred to as the final product in Table II.

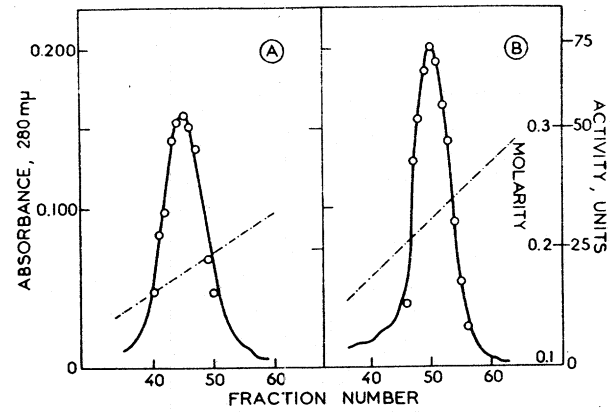

Fig. 4. Rechromatography of acid deoxyribonuclease fractions A and B on CM–Sephadex C-50. 8 $A_{280m\mu}$ units of each acid deoxyribonuclease (preparation HS 10) fractions A and B were loaded on two 1 cm × 100 cm CM–Sephadex C-50 columns. A molarity gradient (0.1–0.4 M) of phosphate buffer (pH 6.8) was started at fraction 1 (right-hand inner scale). 3-ml fractions were collected. The continuous line shows the absorption at 280 mμ. Circles indicate the deoxyribonuclease activity (right-hand outer scale).

Properties of the enzyme

The physical and chemical properties of acid deoxyribonuclease (main or B component; preparation HS 7)have already been reported elsewhere[8]. They were found to be indistinguishable from those of enzyme preparations obtained with the older procedure of BERNARDI AND GRIFFE[2]. The enzymological properties and purity are also identical to those already reported[2].

Comparative study of A and B components

The properties of acid deoxyribonucleases A and B were compared, using in all cases the same amounts and concentrations (as determined from the absorbances at 280 mμ) of the two components in 0.15 M acetate buffer (pH 5.0). The results obtained were the following:

Sedimentation velocity. The A and B components were studied in the analytical ultracentrifuge, using enzyme concentrations in the 0.1–0.5 % concentration range and wedge cells; the sedimentation coefficients were found to be the same. Sucrose-gradient centrifugation was also used with cytochrome *c* as a reference protein; again the sedimentation coefficients of the two components were found to be identical and over 90 % of the loaded activities was recovered in the enzyme bands.

Gel filtration on Sephadex G-100 column (1 cm × 100 cm). This was carried out using Dextran Blue 2000 (Pharmacia, Uppsala, Sweden) and cytochrome *c* as reference markers. The elution volumes of the two activities were identical and a practically complete recovery was obtained for both components.

Enzymological properties. Both the deoxyribonuclease and the phosphodiesterase activities of A and B components were measured at different substrate concentrations, and found to be the same.

Ultraviolet spectra of the two components were indistinguishable.

Carbohydrate contents. The colored product(s) formed by the orcinol reaction[16] on the two components showed the same absorbances at 420 and 540 mμ.

Peptide maps. The tryptic peptides of heat-denatured deoxyribonucleases A and B, when mapped according to KATZ, DREYER AND ANFINSEN[17], showed the same pattern with the only difference that one particular single peptide spot of component A was resolved into two spots in the map of component B*.

Chromatographic behaviour. Single peaks were obtained on running artificial mixtures (50:50) of the two components on DEAE–Sephadex and hydroxyapatite columns in the experimental conditions given above or on Amberlite IRC-50 according to BERNARDI AND GRIFFE[2]. A very poor resolution was obtained on this latter column using greater column length: diameter ratios than those used by BERNARDI AND GRIFFE[2].

The comparative results obtained for fractions A and B of spleen acid deoxyribonuclease indicate that the two products do not differ in their macromolecular and enzymological properties. Component A is likely to be slightly more acidic than component B since it is eluted from a carboxylic ion exchanger by a lower phosphate molarity. The peptide maps of the two fractions suggest that component B has one more peptide bond susceptible of being split by trypsin than component A.

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* G. BERNARDI, work to be published.

These findings may be tentatively explained by admitting that A differs from B in that an asparagine (or a glutamine) residue adjacent to a lysine (or an arginine) has been deamidated by the acidic treatment involved during the preparation of the enzyme, the resulting peptide bond being resistant to trypsin[18].

To test this hypothesis three acid deoxyribonuclease preparations were made using modifications of the procedure described above. In preparation 1, HCl replaced H_2SO_4; in preparation 2, the acidification step to pH 2.5 was omitted and 0.1 M H_2SO_4 was replaced by the same amount of 0.15 M NaCl. Preparation 3 was like preparation 2, except that 0.15 M NaCl replaced 0.05 M H_2SO_4 in the tissue-homogenization step. All these preparations were carried through the chromatographic purification (Procedure C) and the amount of A and B components were estimated from the elution curve obtained in the CM-Sephadex chromatography. Component A, which represents about 20 % of total deoxyribonuclease when the enzyme was prepared according to the usual procedure, decreased to 15–18 % in preparation 1 to 7 % in preparation 2, and was not present in preparation 3.

As a further test of the hypothesis mentioned above, the B component in 0.15 M NaCl was acidified to pH 2.5 by direct addition of 0.05 M H_2SO_4 or by dialysis against 0.15 M NaCl adjusted to pH 2.5 with H_2SO_4. The acidified samples were then dialyzed against 0.05 M phosphate buffer (pH 6.8) and chromatographed on CM-Sephadex. They showed several small inactive peaks in front of the main activity peak.

DISCUSSION

The comparative study of components A and B, the experiments in which the acidification steps involved in the present preparation procedure were modified or omitted altogether, and the chromatographic results obtained with the acidified B component, all are in favor of the idea (*vide supra*) that component A arises from acid deoxyribonuclease B by a deamidation process occurring at low pH. It is known that acid deoxyribonuclease is very rich in amide groups[8] and that deamidation can occur at low pH in a number of proteins[19]. An alternative explanation would be that the two components are different, genetically determined forms of the same protein. In this case one should admit, however, that the A component, in order to be extracted needs a more acidic solution than the B component.

Studies on the amino acid composition of the peptides which differ in the two components are in progress and should eventually lead to an unequivocal answer. Investigations currently made on the carbohydrate moiety of deoxyribonuclease will establish whether or not this part of the molecule is also altered by the acidic treatment (such as is the case for ribonuclease[20]). This latter alternative is favored by the finding that no change seems to affect the neutral sugars of the molecule.

The results presented in Table I show several interesting facts. 0.1 M HCl or 0.05 M H_2SO_4 are evidently more effective than 0.15 M NaCl in extracting acid deoxyribonuclease; this is very probably due to the fact that the contact with the acidic solution is effective in breaking down the subcellular particles to which the enzyme is bound in the cell. It is important to stress that the difference is, at least in part, due to the extraction of exonuclease by the acidic solutions[13].

The acidification step to pH 2.5 is very effective in releasing more acid deoxyribonuclease. This increase is smaller for the extracts obtained with 0.1 M HCl or 0.05 M H_2SO_4, but it should not be forgotten that splenic exonuclease is almost completely inactivated by this step[13]; the real increase in the amount of extracted enzyme is therefore larger than that shown in the table.

The fractionation between 0.4 and 0.8 saturation of $(NH_4)_2SO_4$ seems to permit a very good recovery of enzyme. This is about 20 % lower when the extraction is carried out with 0.15 M NaCl. Some losses occur in the steps between the precipitation at 0.8 saturation of $(NH_4)_2SO_4$ and the beginning of the chromatographic purification. These are mainly responsible for the decrease of the activity per kg of trimmed spleen from 3600 to about 3000 units. It is important to stress that activities given in Table I represent "practical" yields which may be obtained in preparative work.

The chromatographic purification also requires comment. The resolution of enzymatic activities in the DEAE–Sephadex chromatography may be improved by using a lower phosphate molarity and a higher pH in the eluting buffer. It is difficult, however, to keep a good control of pH and ionic strength in this case, and reproducibility of separations is not very good. The main reason for the choice of 0.05 M phosphate buffer (pH 6.8) for this chromatographic step is to avoid such a drawback. The CM–Sephadex step may be improved by a very prolonged washing of the column with 0.1 M phosphate buffer (pH 6.8) after the loading step. In this case rechromatography on CM–Sephadex may become unnecessary.

In conclusion, the present method can in most cases replace the procedures previously used[2]. Its main advantages over the older method, from which it is derived, are its rapidity, its adaptability to large-scale work, and its higher yield of enzyme. As far as this latter point is concerned, the total deoxyribonuclease activity of the crude enzyme preparation is essentially the same in both cases: about 3000 units/kg of trimmed tissue; the specific activity is, however, about 3 times higher in the new preparation procedure. This factor obviously was of decisive importance for simplifying the chromatographic purification. The final yield of 25 % for the final product which can be calculated from the data presented in Table II, taking as 100 % the deoxyribonuclease activity of the crude enzyme, refers only to the processing of the central fractions of the activity peak obtained from each column. A comparable or higher amount of enzyme may be obtained by processing the side fractions. Therefore the overall yield is about twice that obtained with the older procedure[2].

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